

Research Article

Effects of Nitrogen Fertilization and Soil Inoculation of Sulfur-Oxidizing or Nitrogen-Fixing Bacteria on Onion Plant Growth and Yield

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A field experiment was conducted in a newly reclaimed soil at El-Saff region, El-Giza Governorate, Egypt to study the effects of different rates of nitrogen (N: 62 to 248 kg ha⁻¹) with or without soil inoculation of sulfur- (S-) oxidizing bacteria (SoxB) and combined inoculation of SoxB and N-fixing bacteria (NFxB) on yield, quality and nutritional status of onion (*Allium cepa* L., “Giza 20”). Elemental S at 620 kg ha⁻¹ was applied to all treatments. Application of N at 62, 124, and 248 kg ha⁻¹ rates increased onion yield, plant height, and N uptake by 28 to 76%, 32 to 53%, and 61 to 145%, as compared to those of the plants that received no N. Inoculation of SoxB at various N rates increased onion yields by 47 to 69% and N uptake by 76 to 93%, as compared to those of the plants which received the respective rates of N but no SoxB inoculation. Inoculation with SoxB and NFxB increased onion yield by 221%, plant height by 62%, and N uptake by 629%, as compared to those of the plants grown without inoculation and no N applied.

1. Introduction

Recommendation of optimal nitrogen (N) fertilization strategy and improvement of N management efficiency heavily rely on precise evaluation of N status in plant-soil system. For most annual crops, N is generally applied as a base dressing before planting. Prolonged lag time between N application to the soil and its maximum crop uptake, following seedling emergence and rapid growth, may result in N leaching below the rootzone, hence not available for plant uptake. To maximize the benefits of N nutrition and to ensure adequate N availability throughout the growing season, additional N application later during the growing season may be required. Therefore, it is important to follow an optimized N fertilizer application as per recommended or accepted procedures for high yields and quality of onion production [1].

Sulfur (S) is needed by plants and microorganisms, and its speciation in soil is dependent on the chemical state of the soil, including (1) redox potential, that is, tendency of the soil

solution to either gain or lose electrons and thereby suggests the aerobic or anaerobic status of the soil and (2) soil acidity [2]. Plant availability of S in a given soil is dependent on the S speciation in soils, influenced by pedogenetic processes and physicochemical factors, that is, water logging. The oxidation of S to SO₄²⁻ in soil is a biological process and is carried out by several kinds of microorganisms, that is, *Thiobacillus thiooxidans*, *T. ferrooxidans*, *T. thioparus*, *T. denitrificans*, and *T. novellus*. The rate at which this conversion takes place is determined by three main factors, that is, microbiological population in the soil, physical properties of the S source, and environmental conditions. Most agricultural soils contain some microorganisms that are able to oxidize S. However, the most important organisms in S oxidation are a group of bacteria (SoxB) belonging to the genus *Thiobacillus*. The population density of these bacteria generally determines the degree to which S is converted to SO₄²⁻ in soils. Population density of *Thiobacillus* can vary substantially in different soils. Under laboratory conditions, the rate

of S oxidation in some soils can be markedly increased by inoculation with *Thiobacillus* [3]. However, under field conditions, inoculation has not been found very effective. The population of S-oxidizing bacteria increases in the soil following application of S product.

Sulfur (S) is an essential plant nutrient; however, its content in the soil is only about 10% of that of the total N [4]. Therefore, investigations on the S nutrition of plants are rather few, since in most cases, S content in the fertilizers and atmospheric deposition supplies an adequate amount [5].

Reduced S inputs from atmospheric depositions during recent years resulted in a negative S balance in agricultural soils, since crop plants have become increasingly dependent on the soil to supply crop need for S [6]. Insufficient S availability leads to decreased yields and reduced S content in the plant products under extreme deficiency [7]. Most of the S in soils is bound to organic molecules, and therefore not readily available to plants. Use of S oxidizers enhances the rate of natural oxidation of S and production of sulfates and makes them available to plants at their critical stages, resulting in increased plant yield [8]. In microbial oxidation of inorganic S compounds, the reactions often mimic chemical models, but the intermediates formed chemically interact with each other making the pathway complex [9]. The requirement of S mainly differs between crop species and developmental stage of plants. Sulfur requirement is much greater for Sunflower as compared to that for wheat and soybean. The S requirement is quite low during early growth stages for field bean, rice, and maize [10].

The objective of this study was to investigate the effects of varying rates of N (62 to 248 kg ha⁻¹) with or without SoxB, and combined effects of SoxB and nitrogen-fixing bacteria (NFxB) on onion yield, quality, and nutrient uptake on a newly reclaimed soil.

2. Materials and Methods

Field experiment was conducted at El-Saff region, Giza Governorate, South of Cairo, Egypt, located between 29° 38' 25.1'' N and 31° 19' 26.8'' E. Average annual rainfall in this region is only about 2 to 5 mm. During the winter growing season of 2007-2008, average temperature was 13 to 21°C. Wind storms from the south (referred to as "Khamaseen"), usually in spring or summer, brings sand and dust, and sometimes raises the temperature in the desert to more than 38°C. The physical and chemical properties of the surface soil (0–30 cm) determined according to Page et al. [11] and FAO [12] include the revealed contents of sand, clay, and silt as 87.6, 6.8, and 5.6%, respectively. The other properties include organic matter = 0.35%, CaCO₃ = 12.7%, concentrations of available N, P, K, and S were 15.3, 10.6, 65.7, and 7.4 mg kg⁻¹, respectively, with pH = 7.80 and EC = 2.3 dS m⁻¹. Onion (*Allium cepa* L.-cultivar: Giza 20) seedlings were planted 10 cm apart on the two sides of row spaced 0.5 m, with plot area of 3 m² (1.5 × 2 m).

The following treatments were evaluated in a randomized complete block design with three replicates:

- (i) control, that is, soil with no inoculation or N fertilizers (CK),
- (ii), (iii), and (iv) received 62, 124, and 248 kg ha⁻¹ N application, respectively (62N, 124N, and 248N),
- (v) to (vii) are treatments similar to (ii) to (iv) with inoculation of S oxidizing bacteria (SoxB): (62N + SoxB; 124N + SoxB; 248N + SoxB),
- (viii) no N fertilizer, plus inoculation of SoxB and N-fixing bacteria (NFxB): (CK + SoxB + NFxB).

Super phosphate (6.8% P) and potassium sulphate (43% K) were broadcasted at quantities equivalent to 494 and 247 kg ha⁻¹, respectively, and incorporated with soil before planting. Nitrogen fertilizer was applied as ammonium nitrate (33.5% N) in three applications, that is, at sowing, plus two applications at monthly interval. All treatments received elemental sulfur (S) at 620 kg ha⁻¹ and 25 Mg ha⁻¹ organic manure incorporated before planting. Irrigation was done once every 48 hrs by drip system with one drip tape per row and drippers with 4 L h⁻¹ discharge rate spaced at 0.33 m.

2.1. Microorganisms Used and Method of Inoculation. The various microorganisms used in this study include SoxB and NFxB with establishment of their growth parameters. The methodologies used are given below.

2.2. *Thiobacillus Thiooxidans* (Sulfur-Oxidizing Bacteria (SoxB)). *Thiobacillus thiooxidans* strain was isolated and identified at the Agricultural Microbiology Department, National Research Center [13], Cairo, Egypt. Bacterial cultures were grown in 250 mL Erlenmeyer flasks using Starkey's medium [14] at 30°C for 4 weeks on shaker at 220 rpm. The density of bacterial culture in the broth counted using optical density was 10⁸ CFU mL⁻¹ before being used to fortify with organic manure. Inoculation treatments were applied at the rate of 100 mL plot⁻¹ mixed with weighed amount of organic manure and then broadcast uniformly into the plot area.

2.3. Free Nitrogen-Fixing Bacteria (NFxB). Highly efficient strains of plant growth promoting rhizobacteria (PGPR) (*Azotobacter chroococcum* and *Azospirillum lipoferum*) were selected from culture collection of Agricultural Microbiology Department, National Research Center, Cairo, Egypt. *Azospirillum* was maintained at 30°C on malate-yeast extract medium, while *Azotobacter* was kept on Ashby's mannitol agar. The *Azotobacter* and *Azospirillum* were independently grown in nutrient broth for 48 hours at 30°C in a rotary shaking incubator. The density of *Azotobacter* and *Azospirillum* in the broth was 10⁷ and 10⁶ of CFU mL⁻¹, respectively. One hundred mL each of liquid broth cultures of *Azotobacter* and *Azospirillum* initially containing 7 × 10⁸ and 5 × 10⁷ viable cell mL⁻¹, respectively, was mixed with preweighed quantity of organic manure per plot, which was then uniformly distributed over the plot surface area.

2.4. Response Parameters. Soil sample was taken from 0 to 15 cm depth from each plot on 7, 15, 30, 60, 90, and 120

days after planting for analysis of water-soluble sulphate and pH. Plant samples were collected at the maturity stage (150 days) to measure leaf length, fresh and dry weights of leaves and bulbs, and total yield. Concentrations of N, P, K, Zn, Fe, and Mn in plants were determined according to Kalra and Maynard [15].

The data obtained as average of the two successive seasons were subjected to two-way analysis of variance (ANOVA) to test the significance of treatment effects. Test of significance for differences in means was done using least-square difference (LSD) described by Snedecor and Cochran [16].

3. Results and Discussion

3.1. Effects of S Oxidation in Onion Rhizosphere on Some Soil Properties. Oxidation of S in onion rhizosphere was monitored based on SO_4^{2-} concentration at various durations during the plant growing period (Figure 1). The SO_4^{2-} concentration increased with increasing rate of N (62 to 248 kg ha^{-1}). Furthermore, inoculation with SoxB increased the SO_4 concentration across all N rates. Combined inoculation of SoxB and NFxB (with no N applied) did not appear to enhance S oxidation as compared to inoculation of only SoxB. Most agricultural soils contain an abundance of native SoxB population; hence, SoxB inoculation may not be necessary. However, some soils do respond to SoxB inoculation as was the case in this study. Our results agree with that of Kapoor and Mishra [17] who reported rapid oxidation of S in a field soil with pH 8.0, and the rate of oxidation was further enhanced by SoxB inoculation.

The SO_4^{2-} concentration in the soil increased from 7 to 30 days after planting (DAP), by 38, 35 to 56, 52 to 68, and 56%, respectively, in control, 62 to 248 kg ha^{-1} N rate, different N rates plus SoxB inoculation, and SoxB and NFxB inoculation treatments. Subsequently, the SO_4^{2-} concentration decreased until 120 DAP. The corresponding percent reductions for the above treatments between 30 and 120 DAP were 45, 40 to 43, 29 to 30, and 31%. These results suggest that in the soil with SoxB inoculation, the increase in SO_4^{2-} concentration during 7 to 30 DAP was greater and decrease during 30 to 120 DAP was lower as compared to that in the soil without SoxB inoculation. Leaching of SO_4^{2-} below the depth of soil sampling may account, in part, for this reduction in SO_4^{2-} concentration. Net mineralization of S was significantly greater in SoxB-inoculated soils compared to that in noninoculated soils.

3.2. Changes in Soil pH in Onion Rhizosphere. Across most treatments, the soil pH somewhat decreased or remained unchanged from 7 to 30 DAP, followed by gradual increase during 60 and 90 DAP with highest pH values on 120 DAP (Figure 2). The initial decrease in pH values might be ascribed to H^+ ion released during S oxidation. When elemental S is applied to soil, a biological reaction takes place carried out by SoxB, producing sulfuric acid that reduces soil pH [18]. The analysis of field soil samples taken after harvest revealed that soil inoculation of SoxB reduced soil pH from

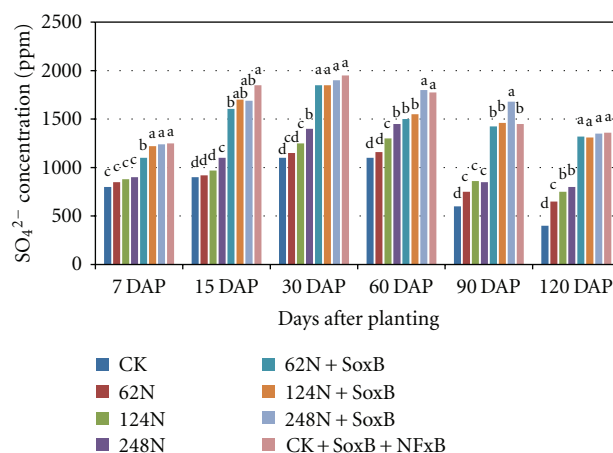


FIGURE 1: Changes in SO_4^{2-} concentration in onion rhizosphere soil over 120 days after planting (DAP). Means followed by the same letters, by each sampling date, are not significantly different as per Duncan's multiple range test at $P \leq 0.05$.

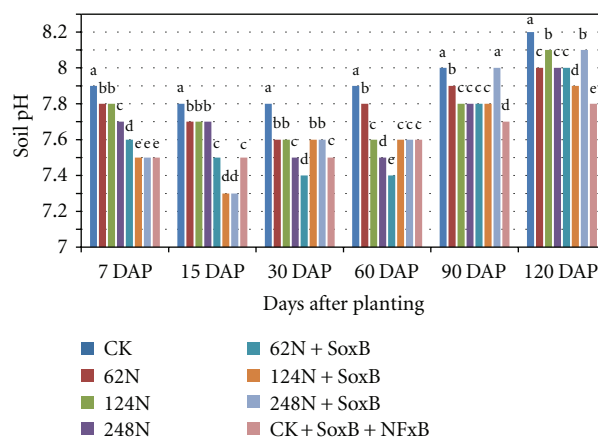


FIGURE 2: Change in soil pH in onion rhizosphere soil over 120 days after planting (DAP). Means followed by same letters, by each sampling date, are not significantly different as per Duncan's Multiple Range test at $P \leq 0.05$.

7.8 to 7.5. Anandham et al. [19] reported that inoculation of SoxB also increased available soil—S from 7.4 to 8.43 kg ha^{-1} , and EC from 0.20 to 0.25 dS m^{-1} . Anandham et al. [19] also reported that the soil pH decreased from 7.2 to 7.0; however, no negative effects were evident on plant growth. A study on peanut production revealed that application of elemental S (0.6 Mg ha^{-1}) and inoculation of *Thiobacillus* significantly reduced the soil pH from 8.2 to 7.1 at the end of the growing period [20]. Decrease in soil pH is desirable in calcareous and saline soils for improved plant growth and adequate micronutrient availability.

3.3. Plant Growth and Yield. Onion plant height increased by 32% with 62 kg ha^{-1} N rate as compared to that grown with no N application (Table 1). Further increment in N rate had no significant influence on the plant height. Soil inoculation with SoxB at different N rates failed to show any beneficial

TABLE 1: Effects of different rates of nitrogen (kg ha^{-1}) without or with inoculation of sulfur-oxidizing bacteria (SoxB) and soil inoculated with SoxB and N-fixing Bacteria (NFxB) on onion plant growth parameters and bulb yield.

Treatments	Plant height (cm)	Onion yield	Fresh bulb	Dry bulb (Mg ha^{-1})	Fresh leaves	Dry leaves
CK	34	13.1	11.1	1.56	1.61	0.37
62N	45	16.8	14.1	2.25	2.35	0.59
124N	49	19.0	15.3	2.35	3.24	0.67
248N	52	23.0	18.8	2.87	3.95	0.91
62N + SoxB	44	28.4	22.7	3.36	4.77	0.96
124N + SoxB	46	30.4	25.2	3.78	5.98	1.01
248N + SoxB	48	33.8	27.9	4.32	6.40	1.09
CK + SoxB + NFxB	55	42.0	34.8	5.58	8.32	1.58
¹ LSD at 0.05	8	4.0	3.0	0.64	1.06	0.02

¹ LSD: least significant difference.

TABLE 2: Effect of nitrogen and soil inoculation with sulfur-oxidizing (SoxB) and nitrogen-fixing bacteria (NFxB) on nutrient uptake by onion plants.

Treatments	Macronutrients uptake (kg ha^{-1})			Micronutrients (ppm)		
	N	P	K	Zn	Fe	Mn
CK	24.9	1.7	24.2	7.5	2.4	15.7
62N	40.0	3.5	32.6	16.7	5.7	27.7
124N	51.4	4.7	39.0	32.9	14.2	35.8
248N	61.0	5.9	49.9	75.0	31.7	37.0
62N + SoxB	77.1	6.4	58.8	78.4	42.9	40.0
124N + SoxB	90.4	7.4	70.1	81.0	44.9	40.3
248N + SoxB	108.7	10.4	85.7	88.8	47.1	42.7
CK + SoxB + NFxB	181.5	14.6	114.9	99.8	56.0	57.1
¹ LSD at 0.05	21.2	1.5	19.3	6.9	8.3	10.8

¹ LSD: least significant difference.

effects on plant height. Height of the plants grown in the soil inoculated with SoxB and NFxB (no N), and the soil received 62 to 248 kg ha^{-1} N rates were greater by 62% and 32 to 53%, respectively, as compared to that of the plants grown in the soil with no N and no inoculation. Highest onion yield was also obtained in the soil inoculated with SoxB and NFxB (Table 1), followed by the soil with 248 kg ha^{-1} N and SoxB inoculation. Inoculation with SoxB significantly increased the onion yield by 47 to 69% across different N rates, as compared to that of the plants that received respective N rates but not SoxB inoculation. The onion yield was the lowest in soil with no inoculation and no N application. Nitrogen and S appear to exert strong effects on various growth parameters of onion plants and flavor biosynthetic pathway [21]. Songzhong et al. [1] investigated the effects of different N supply levels (1.5, 3.0, 6.0, 12.0, and 24.0 mmol L^{-1} of N) in Chinese spring onion (*Allium fistulosum* L. var *giganteum* Makino) in soilless growing media. High N rate (24 mmol L^{-1}) significantly inhibited plant growth, retarded S assimilation, and decreased pungency. However, N rates in the range of 360 to 600 kg N ha^{-1} are common in Zhangqin County, China, despite the recommended rate of only 240 kg N ha^{-1} for a target yield of 60 Mg ha^{-1} [22].

Sulfur-based fertilizers decrease the pH of soil and, thus, increase the uptake of other plant nutrients, which contributes to increased yields. The results of our study are in agreement with that of Sullivan et al. [23], Sutaliya et al. [24], and El-Desuki et al. [25]. Smatanová et al. [26] reported that the application of S as $(\text{NH}_4)_2\text{SO}_4$ had positive effects on yields and quality of the vegetables. Anandham et al. [19] reported that inoculation of both *Thiobacillus* and *Rhizobium* increased the shoot height, root length, and plant biomass on 40 and 80 days after seeding in pot and field experiments as compared to the plants inoculated with single strain. Banerjee and Yesmin [27] discovered that the use of S-oxidizing rhizobacteria in combination with elemental S increased canola yield in S-deficient soils. Thus, they concluded that S-oxidizing rhizobacteria was effective to enhance canola production.

Whipps [28] stated that the importance of plant growth-promoting rhizobacteria (PGPR) was well established and to some extent, the beneficial effects of PGPR may be linked to biocontrol. PGPR increases plant growth indirectly either by the suppression of well-known diseases caused by major pathogens or by reducing the deleterious effects of minor pathogen, that is, microorganisms which reduce plant

growth but without obvious symptoms. Yesmin and Banerjee [29] isolated S oxidizers among the rhizobacterial strains and that these bacteria were critical to meet plant S demand while utilizing elemental S. These S oxidizers had stimulating effect on canola plant emergence. Results indicated that bacterial inoculation enhanced canola biomass and yield. Okon and Labandera-Gonzalez [30] reported wheat yield increase of up to 30% in 60–70% of the trials with S oxidizers. Yield increases have been attributed to mechanisms such as N fixation, phytohormone, and nitrate reduction.

The response to rhizobacteria inoculation depends on different factors, including inoculation, chances for survival and motility, adsorption by soil particles, competition with indigenous populations of rhizobacteria, and soil fertility [31, 32]. Some soil microorganisms, like *Azospirillum* sp. [33], *Enterobacter* sp., *Azotobacter* sp., and *Pseudomonas* sp. [34], have shown to encourage plant growth [35] by promoting the outbreak of secondary roots, acting as protectors against pathogenic microorganisms by release of plant hormones and siderophores [36].

3.4. Nutrients Uptake in Onion Bulbs. Uptake of most nutrients was high in the plants grown in soil inoculated with SoxB and NFxB, followed by that in plants received N rates plus SoxB, only N, and soil without N or inoculation (Table 2). This trend also supports the trend in onion yields and, thus, increased nutrient uptake by a given soil treatment contributed to increased bulb yield (Tables 1 and 2). The reduction in soil pH with inoculation of SoxB and NFxB may have contributed to increased availability of micronutrients which in turn lead to increased micronutrient uptake (Table 2). Concentration of N in onion bulbs was greater in plants grown in soil inoculated with SoxB and NFxB. These results agree with those reported by Rizk [37] and El-Desuki et al. [25] who concluded that adequate N and S availability was critical for increasing the yield.

4. Conclusion

The results of this study demonstrate the beneficial effects of application of N and elemental S along with inoculation of SoxB and NFxB on onion yields. Balanced fertilization is essential to increase yields and net returns while minimizing nutrient loss to the environment. However, more field studies under different agroclimatic conditions are required to confirm the above findings.

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